Yeast Galactose Induction

Introduction

Protocol for induction of protein production using the GAL promoter in yeast.

Materials

- Minimal media with amino acids (expect the one used for selection)
- 20 % raffinose and/or 40 % glucose solution to supplement to medium
- 40 % Galactose solution for induction

Procedure

1. Prepare an overnight preculture by inoculating a single colony into 2-5 mL of approriate selective media. Incubate overnight at 30 C with shaking

   If the protein to be produced is encoded in a plasmid, use knockout mix (minimal media supplemented with amino acids, except for the one you have as an auxotrophic selection marker on your plasmid). Supplement sugar to the media if it doesn't contain it already; either 2 % glucose or 2 % raffinose. Glucose media acheives higher ODs.

2. The next morning, measure OD of your preculture; dilute as necessary to get an accurate measurement and calculate back to get the original OD.

3. Refresh the cell culture; spin cells down (around 3824g, 5 minutes). Remove supernatant. Resuspend cells in selective media containing 2 % raffinose; based on the ODs calculated, add media so that an OD of 0.2 will be acheived.

4. Grow for 4-6 hours at 30 C with shaking. Optimally, an OD of 1 will be acheived.

5. To induce the cultures, add 40 % galactose solution to the culture to acheive a final galactose concentration of 2 % (or alternatively, you can vary between 0.4-4 % as needed)

6. Grow in 30 C with shaking for 20 hours, then harvest cultures.